EBioMedicine 18 (2017) 288–299

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

# Maternal Retinoids Increase PDGFRα$^+$ Progenitor Population and Beige Adipogenesis in Progeny by Stimulating Vascular Development

Bo Wang [a,b], Xing Fu [b], Xingwei Liang [b], Zhixiu Wang [b], Qiyuan Yang [b], Tiande Zou [b], Wei Nie [b], Junxing Zhao [b,e], Pengfei Gao [b,e], Mei-Jun Zhu [c], Jeanene M. de Avila [b], Joseph Maricelli [d], Buel D. Rodgers [b], Min Du [a,b,e,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, China Agricultural University, Beijing, 100194, China
[b] Department of Animal Sciences, Washington State University, Pullman, WA 99164, United States
[c] School of Food Sciences, Washington State University, Pullman, WA, 99164, United States
[d] School of Molecular Biosciences, Washington State University, Pullman, WA 99164, United States
[e] College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, China

ARTICLE INFO

*Article history:*
Received 13 December 2016
Received in revised form 18 March 2017
Accepted 27 March 2017
Available online 4 April 2017

*Keywords:*
Maternal care
Retinoic acid
Vitamin A
Adipose development
Beige adipocyte
Vasculature

ABSTRACT

Maternal vitamin A intake varies but its impact on offspring metabolic health is unknown. Here we found that maternal vitamin A or retinoic acid (RA) administration expanded PDGFRα$^+$ adipose progenitor population in progeny, accompanied by increased blood vessel density and enhanced brown-like (beige) phenotype in adipose tissue, protecting offspring from obesity. Blockage of retinoic acid signaling by either BMS493 or negative RA receptor (RARαDN) over-expression abolished the increase in blood vessel density, adipose progenitor population, and beige adipogenesis stimulated by RA. Furthermore, RA-induced beige adipogenesis was blocked following vascular endothelial growth factor receptor (VEGFR) 2 knock out in PDGFRα$^+$ cells, suggesting its mediatory role. Our data reveal an intrinsic link between maternal retinoid level and offspring health *via* promoting beige adipogenesis. Thus, enhancing maternal retinoids is an amiable therapeutic strategy to prevent obesity in offspring, especially for those born to obese mothers which account for one third of all pregnancies.



## 1. Introduction

With metabolic syndromes such as obesity yielding widespread contemporary concern, the race is on for discovering strategies which can ameliorate conditions detrimental to human health. Nutrigenomics is an emerging field of science aiming to address such conditions by studying the molecular interplay between a diet and the genome. Evidence shows that adipose development at the fetal stage have long term effects in later life. In mice, subcutaneous white fat (WAT) (Wang et al., 2013) and brown fat (BAT) (Billon and Dani, 2012) develop during the fetal stage. Maternal nutritional status during gestation epigenetically alters WAT differentiation (Borengasser et al., 2013). *Zfp423*, a gene encoding a transcription factor for preadipocyte commitment, is hypomethylated in fetuses born to obese mothers (Yang et al., 2013). Moreover, the population of adipose progenitor cells (Liang et al., 2016a) and the thermogenic function of BAT (Liang et al., 2016b) of offspring are altered due to maternal intake of high fat diet. Up to now, the relationship between early adipose development and obesity in later life remains poorly understood.

White adipocytes are healthy at normal sizes, but over-expansion of adipocyte sizes lead to hypoxia (Sun et al., 2011), inflammation, and interstitial fibrosis (Sun et al., 2013), which triggers adipose metabolic dysfunction (Sun et al., 2014b). Beige adipocytes, which distribute inside white adipose tissue, burn fatty acids to reduce adipocyte hypotrophy. The vascular system acts as an adipogenic niche by providing PDGFRα$^+$ progenitors, which are able to differentiate into both beige and white adipocytes (Lee et al., 2012). Adipose tissue are highly vascularized (Cao, 2007), and adipogenesis is spatially and temporally associated with angiogenesis during fetal development (Cao, 2007), which provides PDGFRα$^+$ progenitor cells (Crisan et al., 2008; Tran et al., 2012; Vishvanath et al., 2016). Thus, promoting beige adipogenesis of PDGFRα$^+$ progenitors improves the metabolic health of adipose tissue.

Vitamin A and its metabolite, retinoic acid (RA), play key roles in fetal morphogenesis and organ development (Zile, 2001; Duester, 2008), and is a common supplement used during pregnancy. At pharmacological doses, RA increases energy consumption of white adipose tissue (Alvarez et al., 1995; Puigserver et al., 1996; Bonet et al., 2003; Mercader et al., 2006), suggesting that RA might promote beige adipogenesis in mature WAT. Vitamin A or RA supplementation is effective in preventing obesity in adult mice (Berry et al., 2012; Noy, 2013). In humans, overweight and obese individuals showed lower retinoid

* Corresponding author at: Department of Animal Sciences, Washington State University, Pullman, WA 99164, United States.
*E-mail address:* min.du@wsu.edu (M. Du).

http://dx.doi.org/10.1016/j.ebiom.2017.03.041

levels in serum (de Souza Valente da Silva et al., 2007; Pereira et al., 2012) and dietary vitamin A intake is inversely related with adiposity (Zulet et al., 2008). However, the effect of maternal vitamin A intake on beige adipogenesis during fetal development is unclear, which represents a critical knowledge gap because the fetal and neonatal stages are critical for adipogenesis. The long-term impact of maternal retinoid status on adipose metabolic health of offspring remains unclear. We hypothesized that maternal vitamin A supplementation promotes angiogenesis and beige adipogenesis during fetal development, which improve the metabolic health of offspring adipose tissue, protecting offspring from diet-induced obesity and metabolic dysfunction.

Currently, 190 million preschool-age children and 19.1 million pregnant women worldwide are at risk of vitamin A deficiency (WHO, 2009). This indicates that a large population of people do not have enough vitamin A intake, especially in the low-income countries. Thus, dietary vitamin A supplementation is expected to be beneficial. However, at very high supplementation levels, vitamin A may be harmful (Ritchie et al., 1998). In rodents, a dose of 75,000 IU/day/rat maternal vitamin A intake is teratogenic (Cohlan, 1954). In humans, a daily dose of 10,000 IU or a weekly dose of up to 25,000 IU during pregnancy are considered to be safe (WHO, 1998), which is a quite high threshold compared to the Recommended Dietary Allowance (RDA) for vitamin A at 700 μg/day or about 2333 IU/day during pregnancy (U.S. Department of Agriculture). Therefore, there is a knowledge gap on the effects of vitamin A at levels moderately above normal intake on fetal development. Our data demonstrate that maternal administration of vitamin A and RA during pregnancy and lactation at physiologically relevant levels enhances beige adipogenesis during early development, which protects offspring from diet-induced obesity and metabolic syndrome in later life.

## 2. Material and Methods

### 2.1. Antibodies and Chemicals

Antibodies against β-tubulin (#2146), cytochrome *c* (#4280) were purchased from Cell Signaling (Danvers, MA). Antibodies against UCP1 (Cat. No. PA1-24894) and PRDM16 (Cat. No. PA5-20872) were bought from TheromoFisher Scientific (Waltham, MA). Antibodies against PDGFRα (Cat. No. 1062-PR) and VEGFR2 (Cat. No. AF644) were bought from R&D. Alexa Fluor 488 anti-mouse CD309 (Cat. No. 136408), APC anti-mouse CD140a (Cat. No. 135908), PerCP/Cy5.5 anti-mouse Sca-1 (Cat. No. 108124), PE/Cy7 anti-mouse CD45 (Cat. No. 103114) were bought from Biolegend (San Diego, CA).

1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (42364), Tamoxifen (T5648), all-trans-Retinoic acid (R2625), insulin (I3536), dexamethasone (D4902), 3-isobutyl-1-methylxanthine (I5878), Triiodothyronine (T3) (IRMM469) and Oil-Red O (O0625) were purchased from Sigma (St Louis, MO, USA). BMS493 (Cat. No. 3509) were purchased from Tocris Bioscience (Ellisville, MO). Mouse recombinant VEGF165 (Cat. No. 583106) was purchased from Biolegend. Vitamin A (M4068, retinyl acetate, water soluble) was purchased from MP Biomedicals, LLC.

### 2.2. Mice

All animal studies were conducted in AAALAC-approved facilities and according to protocols approved by the Institutional Animal Care and Use Committee (IACUC). Wild-type (WT) C57BL/6 mice, *Pdgfra*-Cre-ER (stock number: 018280), *Vegfr2*$^{e3loxP/e3loxP}$ (stock number: 018977) and Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo}$ reporter (mT/mG) mice (stock number: 007676) were purchased from the Jackson Laboratory (Bar Harbor, ME). ROSA26-RARαDN mice were provided by Dr. Cathy Mendelsohn (Rosselot et al., 2010). Mice were mated overnight and the day of vaginal plug detection was marked as E0.5. Retinoic acid, BMS493 or vehicle (DMSO) dissolved in corn oil were injected to pregnant mice twice (10 mg/kg BW) at E10.5 and E13.5. Genetic recombination was induced by one injection of 20 mg/kg BW tamoxifen. For the maternal vitamin A supplementation, 6 litters were obtained for each treatment, and the pups in each litter were adjusted to 6 at birth. The pups were weighed at birth (male) and weaning (male). Twelve male pups in each group were euthanized at weaning for sample collection. Tissues from each litter (2 males) were pooled to get 6 samples for qRT-PCR or western blot analysis. Six male offspring in each group were further treated with HFD. For the RA/BMS493 administration during pregnancy, 6 litters were obtained for each treatment, and the pups in each litter were adjusted to 6 at birth. Six weanling male offspring in each group were euthanized for sample collection and further analysis. For the transgenic mouse lines, 6 weanling male mice in each group were euthanized for sample collection and further analysis.

A commercial diet (2018 Teklad Global) containing 15 IU/g Vitamin A (retinyl acetate which is water soluble) was used. In the current study, the pregnancy mice drank about 3 ml/day. The vitamin A provided by water (3000 IU/kg/day) is about twice of the vitamin A content in the diet. This dose is far below the lowest toxicity dose for rodent (~ 163,000 IU/kg/day) (Ritchie et al., 1998). To avoid oxidation, vitamin A solution was changed daily.

### 2.3. Tissue Processing and Histology

For adipose tissue structure and immunostaining, tissues were fixed in 4% paraformaldehyde (PFA) for 12 h at 4 °C then used for paraffin embedding, sectioning and H&E or immunostaining. For immunostaining, sections were heated in citrate buffer for 20 min, blocked with 5% goat serum in TBS containing 0.3% Triton X-100 for 2 h, then incubated sequentially with primary antibodies overnight and secondary antibodies for 1 h. Sections were then mounted in a mounting medium (Vector Lab, Burlingame, CA). H&E stained sections were used for measuring adipocyte size and number using Image J. Blood vessels were labeled according to a published protocol (Li et al., 2008). Briefly, mice were euthanized by $CO_2$, then sequentially perfused with PBS, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) and 4% PFA into left ventricle (the right atrium was open). Tissues were then sectioned for further whole mount staining or imaging. For whole mount staining, tissues were blocked with 5% goat serum in TBS containing 1% Triton X-100 and 0.2% sodium azide for 2 h, then incubated sequentially with primary antibodies for 4 days and secondary antibodies for 2 days. The PDGFRα tracking mice were perfused with PBS and 4% PFA after euthanized by $CO_2$. Tissues were then sectioned for direct examination and imaging under a Leica TCS SP8 confocal microscope, or tissues were used for further whole mount staining. The confocal microscope images were processed to create 3D Videos using Fiji Imaging Processing Package (Schindelin et al., 2012).

### 2.4. Isolation of Adipose-Derived Stromal Vascular Cells (SVCs)

Inguinal and epididymal fats were isolated, then washed with phosphate-buffered saline (PBS). Tissues were cut into small pieces and digested in digestion buffer containing 0.75 U/ml collagenase D (Roche, Pleasanton, CA) and 1.0 U/ml Dipase type II (Roche) for 30 min at 37 °C. The lysate was filtered sequentially through 100 μm cell strainers, then centrifuged for 5 min at 500*g*. The precipitated cells were then re-suspended and seeded in culture plates.

### 2.5. Oxygen Consumption Assay

Oxygen consumption was measured using a Thermo Scientific Orion 3-Star Dissolved Oxygen meter (Thermo Electron Corporation, Madison, WI). Fresh culture medium was added to the plates, and the dissolved oxygen concentration was measured at the start and after 30 min incubation.